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TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5) 10/088677 Unassigned
INTERNATIONAL APPLICATION NO. PCT/GB00/03601	INTERNATIONAL FILING DATE 20 September 2000	PRIORITY DATE CLAIMED 21 September 1999
TITLE OF INVENTION USE OF REPLICATION-DEFICIENT ADENOVIRAL VECTOR TO BOOST CD8+T CELL IMMUNE RESPONSE TO ANTIGEN		
APPLICANT(S) FOR DO/EO/US SCHNEIDER et al		
<p>Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:</p> <ol style="list-style-type: none"> <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371. <input checked="" type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below. <input checked="" type="checkbox"/> The U.S. has been elected by the expiration of 19 months from the priority date (Article 31). A copy of the International Application as filed (35 U.S.C. 371(c)(2)). <ol style="list-style-type: none"> <input type="checkbox"/> is attached hereto (required only if not communicated by the International Bureau). <input checked="" type="checkbox"/> has been communicated by the International Bureau. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US). <input type="checkbox"/> An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)). <ol style="list-style-type: none"> <input type="checkbox"/> is attached hereto. <input type="checkbox"/> has been previously submitted under 35 U.S.C. 154(d)(4). <input type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)). <ol style="list-style-type: none"> <input type="checkbox"/> are attached hereto (required only if not communicated by the International Bureau). <input type="checkbox"/> have been communicated by the International Bureau. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. <input type="checkbox"/> have not been made and will not be made. <input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)). <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). <input type="checkbox"/> A English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)). <p>Items 11 To 20 below concern document(s) or information included:</p> <ol style="list-style-type: none"> <input checked="" type="checkbox"/> An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. 3.28 and 3.31 is included. <input checked="" type="checkbox"/> A FIRST preliminary amendment. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment. <input type="checkbox"/> A substitute specification. <input type="checkbox"/> A change of power of attorney and/or address letter. <input type="checkbox"/> A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821-1.825. <input type="checkbox"/> A second copy of the published international application under 35 U.S.C. 154(d)(4). <input type="checkbox"/> A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4). <input type="checkbox"/> Other items or information. 		

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

SCHNEIDER et al

Atty. Ref.: 620-190

Nat'l Phase Int'l Appln. No. PCT/GB00/03601

Group:

Int'l Filing Date: 20 September 2002

Examiner:

For: USE OF REPLICATION-DEFICIENT ADENOVIRAL VECTOR TO BOOST CD8+T
CELL IMMUNE RESPONSE TO ANTIGEN

* * * * *

March 21, 2002

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

PRELIMINARY AMENDMENT

Please preliminarily amend the above-identified application
as follows:

IN THE CLAIMS

Please cancel claims 1-8 without prejudice.

Please substitute the following amended claims for
corresponding claims previously presented. A copy of the
amended claims showing current revisions is attached.

SCHNEIDER, et al

Nat'l Phase Int'l Appln. No. PCT/GB00/03601

14. (Amended) A method according to claim 9 further comprising administration of another, different boosting composition comprising said antigen or epitope.

15. (Amended) A method according to claim 9 wherein the boosting composition is administered intradermally.

16. (Amended) A method according to claim 9 wherein the boosting composition is administered intramuscularly.

REMARKS

The above amendments have been made to place the application in a more traditional format.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "**Version With Markings To Show Changes Made.**"

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADEIN THE CLAIMS

14. (Amended) A method according to [any one of claims 9 to 13] claim 9 further comprising administration of another, different boosting composition comprising said antigen or epitope.

15. (Amended) A method according to [any one of claims 9 to 14] claim 9 wherein the boosting composition is administered intradermally.

16. (Amended) A method according to [any one of claims 9 to 14] claim 9 wherein the boosting composition is administered intramuscularly.

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USE OF REPLICATION-DEFICIENT ADENOVIRAL VECTOR
TO BOOST CD8+ T CELL IMMUNE RESPONSE TO ANTIGEN

The present invention relates to generation of a CD8+ T cell
5 immune response against an antigen. More particularly, the
present invention relates to "prime and boost" immunisation
regimes in which the immune response induced by administration
of a priming composition is boosted by administration of a
boosting composition. The present invention is based on the
10 inventors' experimental demonstration that effective boosting
can be achieved using replication-defective adenovirus vectors,
following priming with any of a variety of different types of
priming compositions.

15 A major protective component of the immune response against a
number of pathogens is mediated by T lymphocytes of the CD8+
type, also known as cytotoxic T lymphocytes (CTL). An
important function of CD8+ cells is secretion of gamma
interferon (IFN γ), and this provides a measure of CD8+ T cell
20 immune response.

CD8+ T cell response is important in protection against a
number of parasites, including protozoan parasites such as
Toxoplasma and *Trypanosoma*, *Plasmodium falciparum* (and in mice
25 *P. berghei*), viruses such as HIV, herpes simplex, herpes
zoster, HBV, HCV influenza, EBV, measles, dengue and HTLV-1,

bacteria such as *Mycobacterium tuberculosis* and *Listeria* sp, and various cancers, such as melanoma and renal carcinoma.

Infection of mice with *P. berghei* provides a good model for *P.*
5 *falciparum* malaria in humans, chosen by the inventors for exemplification of the ability of the present invention to provide strong CD8+ T cell immune responses against antigen. Malaria is a major health problem in the world and significant efforts have been directed in finding effective immune
10 compositions for vaccination.

In order to protect against the pre-erythrocytic stage of *Plasmodium falciparum* malaria, an immunogenic composition must induce a strong CD8+ T cell response. In general, live
15 attenuated vaccines capable of producing a short-lived infection that is harmless in a healthy individual are effective at inducing T cell responses. Irradiated *Plasmodium* sporozoites, which can infect hepatocytes but do not progress to a blood-stage infection have been shown to protect both mice
20 and men against malaria by inducing T cell responses against pre-erythrocytic antigens [Nardin and Nussenzweig (1993) Annu. Rev. Immunol. 11: 687-727]. However in man this requires multiple immunisations over a long period of time, and whilst these experimental immunisations have provided valuable
25 information, this approach is not useful for development of a vaccine.

Recombinant viruses can also be used as vaccines. Modified Vaccinia virus Ankara (MVA) does not replicate in human cells and is a very safe virus to use as a vaccine [Mayr et al., (1978) Zentralbl. Bakteriol. 167(5-6): 375-90; Sutter and Moss (1992) Proc. Natl. Acad. Sci. 89(22): 10847-51; Sutter et al., (1994) Vaccine 12(11): 1032-40]. Recombinant MVA expressing *P. berghei* CS has also been tested in mice, resulting in similar levels of peptide-specific lysis in a cytotoxicity assay as mice immunised with Ty VLPs [Gilbert et al., (1999) Biol. Chem.

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380(3): 299-303]. Again, these mice were not protected against infectious challenge. However, despite the fact that neither DNA vaccines, Ty VLPs or MVA used alone can protect against malaria infection, using either DNA or Ty VLPs to prime a T cell response and MVA to boost it results in greatly increased numbers of IFN- γ secreting CD8+ T cells, and complete protection against infection when the MVA is administered intravenously [Schneider et al., (1998) Nat. Med. 4(4): 397-402, WO98/56919].

10

The present invention employs replication-deficient adenovirus which, as the experiments described below show, has been found to be an effective means for providing a boost to a CD8+ T cell immune response primed to antigen using any of a variety of different priming compositions.

15

Replication-deficient adenovirus derived from human serotype 5 has been developed as a live viral vector by Graham and colleagues [Graham and Prevec (1995) Mol. Biotechnol. 3(3): 207-20; Bett et al., (1994) Proc. Natl. Acad. Sci. USA 91(19): 8802-6]. Adenoviruses are non-enveloped viruses containing a linear double stranded DNA genome of around 3600 bp.

20

Recombinant viruses can be constructed by *in vitro* recombination between an adenovirus genome plasmid and a shuttle vector containing the gene of interest together with a strong eukaryotic promoter, in a permissive cell line which

25

allows viral replication. High viral titres can be obtained from the permissive cell line, but the resulting viruses, although capable of infecting a wide range of cell types, do not replicate in any cells other than the permissive line, and are therefore a safe antigen delivery system. Recombinant adenoviruses have been shown to elicit protective immune responses against a number of antigens including tick-borne encephalitis virus NS1 protein [Jacobs et al., (1992) J. Virol. 66(4): 2086-95] and measles virus nucleoprotein [Fooks et al., (1995) Virology 210(2): 456-65]. Further, a single dose of recombinant adenovirus resulted in a 93% decrease in the level of hepatic parasite rRNA of *P. yoelii* in mice, and 40% protection, which was shown to be CD8+ T cell-mediated [Rodrigues et al., (1997) J. Immunol. 158(3): 1268-74].

15

Remarkably, the experimental work described below demonstrates that use of embodiments of the present invention allows for recombinant replication-defective adenovirus expressing an antigen (specifically exemplified with the CS gene from *Plasmodium berghei*) to boost a CD8+ T cell immune response primed by a DNA vaccine, Ty-VLPs or recombinant modified Vaccinia virus Ankara (MVA). The replication-defective adenovirus was found to induce a CD8+ T cell response after intradermal or intramuscular immunisation. In prime/boost vaccination regimes the replication-defective adenovirus was also able to prime a response that could be boosted by MVA.

25

Mice immunised with the replication-defective adenovirus and boosted with the MVA were completely protected against *P. berghei* sporozoite challenge. Both recombinant replication-deficient adenovirus and recombinant MVA are vaccines that are
 5 safe for use in humans. Advantageously, the inventors found that a vaccination regime used intradermal immunisation for both prime and boost can be employed, constituting a general immunisation regime suitable for inducing CD8+ T cells, e.g. in humans.

10

The present invention in various aspects and embodiments employs a replication-deficient adenovirus vector encoding an antigen for boosting a CD8+ T cell immune response to the antigen primed by previous administration of the antigen or
 15 nucleic acid encoding the antigen.

A general aspect of the present invention provides for the use of a replication-deficient adenoviral vector for boosting a CD8+ T cell immune response to an antigen.

20

One aspect of the present invention provides a method of boosting a CD8+ T cell immune response to an antigen in an individual, the method including provision in the individual of a replication-deficient adenoviral vector including nucleic
 25 acid encoding the antigen operably linked to regulatory sequences for production of antigen in the individual by

expression from the nucleic acid, whereby a CD8+ T cell immune response to the antigen previously primed in the individual is boosted.

- 5 An immune response to an antigen may be primed by immunisation, by infection with an infectious agent, or by development of a tumour or malignancy.

A further aspect of the invention provides a method of inducing
10 a CD8+ T cell immune response to an antigen in an individual, the method comprising administering to the individual a priming composition comprising the antigen or nucleic acid encoding the antigen and then administering a boosting composition which
15 comprises a replication-deficient adenoviral vector including nucleic acid encoding the antigen operably linked to regulatory sequences for production of antigen in the individual by expression from the nucleic acid.

A further aspect provides for use of a replication-deficient
20 adenoviral vector, as disclosed, in the manufacture of a medicament for administration to a mammal to boost a CD8+ T cell immune response to an antigen. Such a medicament is generally for administration following prior administration of a priming composition comprising the antigen.

25

The priming composition may comprise any viral vector, although

The priming composition may be a recombinant Ty-VLP. These are
25 protein particles consisting of a single protein species from
the Ty1 retrotransposon of *S. cerevisiae* which spontaneously

In particular embodiments of the various aspects of the present invention, administration of a priming composition is followed by boosting with first and second boosting compositions, the first and second boosting compositions being different from one another, e.g. as exemplified below. Still further boosting compositions may be employed without departing from the present invention. In one embodiment, a triple immunisation regime employs DNA, then adenovirus as a first boosting composition, and then MVA as a second boosting composition, optionally followed by a further (third) boosting composition or subsequent boosting administration of one or other or both of the same or different vectors. Another option is DNA then MVA then Ad, optionally followed by subsequent boosting

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administration of one or other or both of the same or different vectors.

The antigen to be included in respective priming and boosting
 5 compositions (however many boosting compositions are employed) need not be identical, but should share at least one CD8+ T cell epitope. The antigen may correspond to a complete antigen in a target pathogen or cell , or a fragment thereof. Peptide epitopes or artificial strings of epitopes may be employed,
 10 more efficiently cutting out unnecessary protein sequence in the antigen and encoding sequence in the vector or vectors. One or more additional epitopes may be included, for instance epitopes which are recognised by T helper cells, especially epitopes recognised in individuals of different HLA types (such
 15 as tetanus epitopes).

Within the replication-deficient adenoviral vector, regulatory sequences for expression of the encoded antigen will include a promoter. By "promoter" is meant a sequence of nucleotides
 20 from which transcription may be initiated of DNA operably linked downstream (i.e. in the 3' direction on the sense strand of double-stranded DNA). "Operably linked" means joined as part of the same nucleic acid molecule, suitably positioned and oriented for transcription to be initiated from the promoter.
 25 DNA operably linked to a promoter is "under transcriptional initiation regulation" of the promoter. Other regulatory

Administration of the boosting composition is generally about 10 days to 4 weeks after administration of the priming composition, preferably about 2-3 weeks.

beneficial effect.

Most likely, administration will have prophylactic aim to generate an immune response against a pathogen or disease
5 before infection or development of symptoms.

Diseases and disorders that may be treated or prevented in accordance with the present invention include any noted already above and others in which a CD8+ T cell immune response may
10 play a protective or therapeutic role.

Components to be administered in accordance with the present invention may be formulated in pharmaceutical compositions. These compositions may comprise a pharmaceutically acceptable
15 excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material may depend on the route of administration, e.g.
20 intravenous, cutaneous or subcutaneous, nasal, intramuscular, intraperitoneal routes.

As noted, administration is preferably intradermal, subcutaneous or intramuscular.

25

Liquid pharmaceutical compositions generally include a liquid

carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included.

For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required.

A slow-release formulation may be employed.

Following production of replication-deficient adenoviral particles and optional formulation of such particles into compositions, the particles may be administered to an individual, particularly human or other primate.

Administration may be to another mammal, e.g. rodent such as mouse, rat or hamster, guinea pig, rabbit, sheep, goat, pig, horse, cow, donkey, dog or cat.

Administration is preferably in a "prophylactically effective amount" or a "therapeutically effective amount" (as the case may be, although prophylaxis may be considered therapy), this being sufficient to show benefit to the individual. The actual
 5 amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors, or in a veterinary
 10 context a veterinarian, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in
 15 Remington's Pharmaceutical Sciences, 16th edition, Osol, A. (ed), 1980.

In one preferred regimen, DNA is administered (preferably intramuscularly) at a dose of 0.5 mg/injection, followed by
 20 adenovirus (preferably intramuscularly or intradermally) at a dose of 5×10^7 - 5×10^8 virus particles/injection.

A composition may be administered alone or in combination with other treatments, either simultaneously or sequentially
 25 dependent upon the condition to be treated.

Delivery to a non-human mammal need not be for a therapeutic purpose, but may be for use in an experimental context, for instance in investigation of mechanisms of immune responses to an antigen of interest, e.g. protection against cancers,
5 malaria, other pathogens and so on.

Further aspects and embodiments of the present invention will be apparent to those of ordinary skill in the art, in view of the above disclosure and following experimental
10 exemplification, included by way of illustration and not limitation, and with reference to the attached figures, wherein:

Figure 1 shows results of experiments demonstrating peptide-
15 specific IFN- γ secreting T cells primed by a single immunisation of Ad-PbCS. Groups of three mice were immunised via the routes shown using 10^7 pfu. Elispot assays to detect IFN- γ secreting pb9-specific T cells were performed in duplicate on splenocytes after two weeks. The graph shows spot
20 forming cells (SFC) per million splenocytes for each route of administration.

Figure 2 shows results of prime/boost immunisations. Groups of three mice were immunised on day 0 with the first vaccine shown
25 (D = pSG2.PbCS, A = Ad-PbCS, M = MVA-PbCS), and day 14 with the second vaccine. DNA was injected i.m., adenovirus and MVA i.d.

Elispots were performed on splenocytes isolated on day 28. The graph shows SFC per million splenocytes for each prime/boost immunisation regime.

5 Figure 3 shows results of triple combination immunisations, and comparison with double combination immunisations. Groups of three mice were immunised at 10 day intervals with the vaccines shown (D = pSG2.PbCS, A = Ad-PbCS, M = MVA-PbCS), with the first vaccine of the double combinations given on the same day
10 as the second vaccine of the triple combinations. Elispot assays were performed in duplicate on splenocytes 10 days after the last immunisation. The graph shows SFC per million splenocytes for each immunisation regime.

15 EXPERIMENTAL EXEMPLIFICATION

The inventors constructed a recombinant replication deficient adenovirus expressing the CS gene of *P. berghei*, (Ad-PbCS) and tested the capabilities of this virus to induce CD8+ T cell
20 responses in mice either alone or in combination with other types of vaccines.

When used as a single immunisation high levels of antigen-specific CD8+ T cells were generated. Adenovirus priming
25 followed by MVA boosting resulted in complete protection.

Remarkably, adenovirus was able to boost substantially a response primed by DNA, Ty-VLPs or MVA.

MATERIALS AND METHODS

5

DNA vaccine

The DNA vaccine pSG2.PbCS consists of the CMV promoter with intron A driving expression of *P. berghei* CS protein, with the bovine growth hormone poly A sequence. The plasmid is
10 kanamycin resistant and incapable of replication in eukaryotic cells. Plasmids were prepared using Qiagen columns and diluted in endotoxin-free phosphate buffered saline (PBS).

Construction of recombinant replication deficient adenovirus

15 The CMV promoter with intron A, *P. berghei* CS protein gene and bovine growth hormone poly A sequence from pSG2.PbCSP was ligated into the multiple cloning site of the adenovirus shuttle vector pΔE1splA [Bett et al., (1994) Proc. Natl. Acad. Sci. USA 91(19): 8802-6]. This vector can be used to construct

20 Adenovirus 5 recombinants with deletions in E1. The recombinant shuttle vector was used to transfect the permissive cell line 293 along with the adenovirus genome plasmid pJM17 [Graham and Prevec (1995) Mol. Biotechnol. 3(3): 207-20].

Virus from transfected cells was clonally purified by three

25 successive limiting dilutions in 293 cells, and expression of *P. berghei* CS in cells infected with the isolated virus was

confirmed by immunofluoresence. Large quantities of virus were prepared from infected 293 cells and purified by extraction with Arklone [Graham and Prevec (1995) Mol. Biotechnol. 3(3): 207-20] prior to immunisation.

5

Ty VLPs

Recombinant Ty VLPs expressing the pb9 epitope from *P. berghei* CS, SYIPSAEKI, were prepared as described in [Gilbert et al., (1997) Nat. Biotechnol. 15(12): 1280-4] and suspended in PBS.

10

Recombinant MVA

MVA expressing *P. berghei* CS was prepared by *in vitro* recombination between a shuttle vector containing the CS gene driven by the vaccinia P7.5 promoter and MVA virus in primary chick embryo fibroblasts [Sutter et al., (1994) Vaccine 12(11): 1032-40]. The recombinant, which also expresses *E. coli* β -galactosidase, was repeatedly plaque purified and expression of the recombinant gene was confirmed by immunofluoresence. Virus for immunisation was purified by ultracentrifugation through a sucrose cushion and suspended in endotoxin-free PBS.

20

Immunisations

Female BALB/c mice 4-6 weeks old were immunised under anaesthesia as described for individual experiments.

25

Intramuscular DNA immunisations used 50 μ g DNA in each musculus tibialis. MVA and adenovirus (10^6 and 10^7 pfu per dose

respectively) were injected intradermally into the ear pinnae. Ty VLPs (100 µg per dose) were injected intradermally in the footpad or intravenously into the lateral tail vein.

5 *ELISPOT assays*

The number of IFN-γ secreting, pb9-specific T cells in fresh splenocyte preparations was determined as described previously [Schneider et al., (1998) Nat. Med. 4(4): 397-402] by coating 96-well nitrocellulose plates with anti-mouse IFN-γ antibody
10 (clone R4 from ETCC), washing with PBS and subsequent blocking with complete medium containing 10% FCS. Splenocytes from immunised mice were resuspended at $1-2 \times 10^7$ cells/ml and placed in duplicates into the coated wells, and serially diluted. The H2-K^d-restricted peptide pb9 (SYIPSAEKI)
15 (Romero) was added to test wells and an irrelevant peptide to control wells. After overnight incubation the wells were washed and a second, biotinylated anti-IFN-γ antibody (Pharmingen clone) added to the wells. The wells were washed again and streptavidin-alkaline phosphatase was added. After
20 further washing, spots were developed by adding an alkaline phosphatase substrate. The reaction was stopped by washing the wells and spots were counted under a stereomicroscope.

P. berghei challenge

25 Sporozoites of *P. berghei* (ANKA strain clone 1) were obtained

from laboratory reared female *Anopheles stephensi* mosquitoes maintained at 18 °C for 20-25 days after feeding on infected mice. Salivary glands from the mosquitoes were collected by dissection and placed in a tissue homogeniser with RPMI 1640 (Sigma) to release the sporozoites, which were then counted using a haemocytometer. Mice were challenged by injection of 2000 sporozoites into the tail vein. Infection was determined by the presence of ring forms in Giemsa stained blood smears taken 7 and 9 days post challenge. If blood-stage parasitaemia was observed at two time points the mice were sacrificed. Surviving animals were observed for at least a further three weeks for the development of malaria symptoms.

RESULTS

15

Immunogenicity of single adenovirus immunisations using different routes of administration

Initially the effect of the route of administration on the ability of Ad-PbCS to induce pb9-specific IFN- γ secreting T cells was tested.

20

Rodrigues et al. (1997) J. Immunol. 158(3): 1268-74 had found high levels of malaria-specific CD8+ T cells were induced after i.m. and s.c. immunisation, but not i.v., i.p. or i.n. The inventors did not test i.v. or i.p. as these are not suitable routes for a prophylactic vaccine to be used in humans, but

25

included i.d. and "gene paint" groups - simple administration of recombinant adenovirus onto the skin (known as gene painting), previously shown to be able to induce an immune response against the antigen expressed by the virus [Tang et al., (1997) Nature 388(6644): 729-30].

Groups of mice received a single immunisation of 10^7 pfu Ad-PbCS and splenocytes were tested for peptide-specific IFN- γ secreting T cells after 14 days.

10

The numbers of peptide-specific IFN- γ secreting T cells (Figure 1) detected after i.m. or i.d. immunisation were somewhat higher than those detected after a single immunisation with i.m. DNA, and slightly lower than those detected after i.m. MVA [Gilbert et al., (1999) Biol. Chem. 380(3): 299-303].

15

Intranasal or s.c. immunisation produced very low numbers of peptide-specific IFN- γ secreting T cells, and none could be detected in the "gene-paint" group. Intradermal immunisation was used for all subsequent experiments.

20

Immunogenicity of different prime/boost immunisations

25

Figure 2 shows the numbers of peptide-specific IFN- γ secreting T cells detected in the spleens of immunised mice receiving a priming immunisation on day 0 and a boosting immunisation on

day 14.

Using the same vaccine to prime and boost resulted in an increase in specific CD8+ T cells, but a far greater increase was seen after heterologous boosting. DNA does not boost an existing response. However the combination of adenovirus

5 priming and MVA boosting resulted in extremely high numbers of peptide specific CD8+ T cells. In addition to being able to prime a response that could be boosted to such high levels, Ad-PbCS was able to boost a response that had been primed by DNA or MVA.

10

Immunogenicity of triple combination immunisations

Heterologous priming and boosting is clearly much more effective than using the same vaccine repeatedly. Three different vaccines were employed sequentially. DNA vaccines do not boost, so two combinations were possible, and employed: DNA/Ad/MVA and DNA/MVA/Ad. Groups of mice were immunised at 10 day intervals and splenocytes were tested 10 days after the final immunisation.

20 As in the previous experiment high numbers of peptide-specific
IFN- γ secreting T cells were detected after DNA/MVA, Ad/MVA,
MVA/Ad and DNA/Ad immunisations (Figure 3). However these
numbers were not increased to the same extent (three to ten-
fold) when a third heterologous boosting immunisation was
25 given.

Protection against infectious challenge

Several of the prime/boost combinations employing adenovirus as either a priming or boosting agent had resulted in high numbers of peptide-specific IFN- γ secreting T cells that would be

5 expected to protect mice against challenge with *P. berghei* sporozoites. Therefore groups of eight to eleven mice were immunised with a number of different heterologous prime/boost combinations and challenged with 2000 infectious *P. berghei* sporozoites two weeks after the boosting immunisation. The
10 results are shown in Table 1.

Intradermal administration of adenovirus followed by i.d. MVA completely protected the immunised mice. In earlier *P. berghei* challenge experiments it was found that i.m. DNA followed by
15 i.d. MVA gave a high level of protection, but that complete protection was only achieved when the MVA was administered intravenously [Schneider et al., (1998) Nat. Med. 4(4): 397-402]. However using adenovirus priming and MVA boosting, both vaccines could be given intradermally with no loss of
20 protection. MVA priming and adenovirus boosting also resulted in a high level of protection, whereas two subsequent adenovirus immunisations did not. Adenovirus also boosted responses primed by DNA or Ty-VLPs resulting in levels of protection comparable to that obtained by DNA priming and i.d.
25 MVA boosting.

TABLE 1

Protection of mice immunised with different heterologous prime/boost combinations. DNA was given intramuscularly (50 µg dose). MVA (10⁶ ffu dose) adenovirus (10⁷ pfu dose) and Ty VLPs (100 µg dose) were administered intradermally unless otherwise stated. The priming immunisation was given on day 0, the boosting immunisation on day 14, and the challenge carried out on day 28.

10

	Prime	Boost	No.	No.	%
			infected	Challenged	Protection
15	DNA	MVA	5	10	50
	Ad	MVA	0	10	100
	MVA	Ad	2	10	80
	Ad	Ad	7	8	13
	DNA	Ad	5	11	55
20	MVA	MVA	5	8	38
	Ty (i.v.)	Ad	7	10	30
	Ty (i.v.)	MVA (i.v.)	1	11	91
	Ty	Ad	4	10	60
	naïve		8	10	20

ART 34 AMGT

CLAIMS

1. Use of a replication-deficient adenoviral vector encoding an antigen or a CD8+ T cell epitope of said antigen, in the manufacture of a medicament for treating an individual in which a CD8+ T cell immune response to the antigen is of therapeutic or prophylactic benefit, wherein the medicament is for administration to such an individual to boost in the individual a CD8+ T cell immune response to the antigen following prior administration of a non-adenoviral priming composition comprising said antigen or epitope or nucleic acid encoding said antigen or epitope.
2. Use according to claim 1 wherein the priming composition comprises DNA encoding said antigen or epitope.
3. Use according to claim 1 wherein the priming composition comprises recombinant Ty-VLP
4. Use according to claim 1 wherein the priming composition comprises Modified Virus Ankara (MVA).
5. Use according to any one of claims 1 to 4 wherein the medicament is a boosting composition for administration prior to administration of another, different boosting composition comprising said antigen or epitope.

6. Use according to any one of claims 1 to 4 wherein the medicament is a boosting composition for administration following administration of another, different boosting comprising said antigen or epitope.

7. Use according to any one of claims 1 to 6 wherein the medicament is for intradermal administration.

8. Use according to any one of claims 1 to 6 wherein the medicament is for intramuscular administration.

9. A method of boosting a CD8+ T cell immune response to an antigen in an individual, the method including provision in the individual of a replication-deficient adenoviral vector including nucleic acid encoding the antigen or a CD8+ T cell epitope of said antigen operably linked to regulatory sequences for production of said antigen or epitope in the individual by expression from the nucleic acid, whereby a CD8+ T cell immune response to the antigen previously primed with a non-adenoviral vector in the individual is boosted.

10. A method of inducing a CD8+ T cell immune response to an antigen in an individual, the method comprising administering to the individual a priming composition comprising the antigen or a CD8+ T cell epitope of said antigen or nucleic acid encoding said antigen or epitope and then administering a

boosting composition which comprises a replication-deficient adenoviral vector including nucleic acid encoding said antigen or epitope operably linked to regulatory sequences for production of said antigen or epitope in the individual by expression from the nucleic acid.

11. A method according to claim 10 wherein the priming composition comprises DNA encoding said antigen or epitope.

12. A method according to claim 10 wherein the priming composition comprises recombinant Ty-VLP

13. A method according to claim 10 wherein the priming composition comprises Modified Virus Ankara (MVA).

14. A method according to any one of claims 9 to 13 further comprising administration of another, different boosting composition comprising said antigen or epitope.

15. A method according to any one of claims 9 to 14 wherein the boosting composition is administered intradermally.

16. A method according to any one of claims 9 to 14 wherein the boosting composition is administered intramuscularly.

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(54) Title: USE OF REPLICATION-DEFICIENT ADENOVIRAL VECTOR TO BOOST CD8+ T CELL IMMUNE RESPONSE TO ANTIGEN

(57) Abstract: Use of a replication-deficient adenoviral vector encoding an antigen or a CD8+ T cell epitope of the antigen to boost in the individual a CD8+ T cell immune response to the antigen following prior administration of a priming composition. The priming composition comprises the antigen or epitope or nucleic acid encoding the antigen or epitope, and may be DNA, Ty-LVP'S or Modified Virus Ankara (MVA). Administration may be intradermal or intramuscular.

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Figure 1

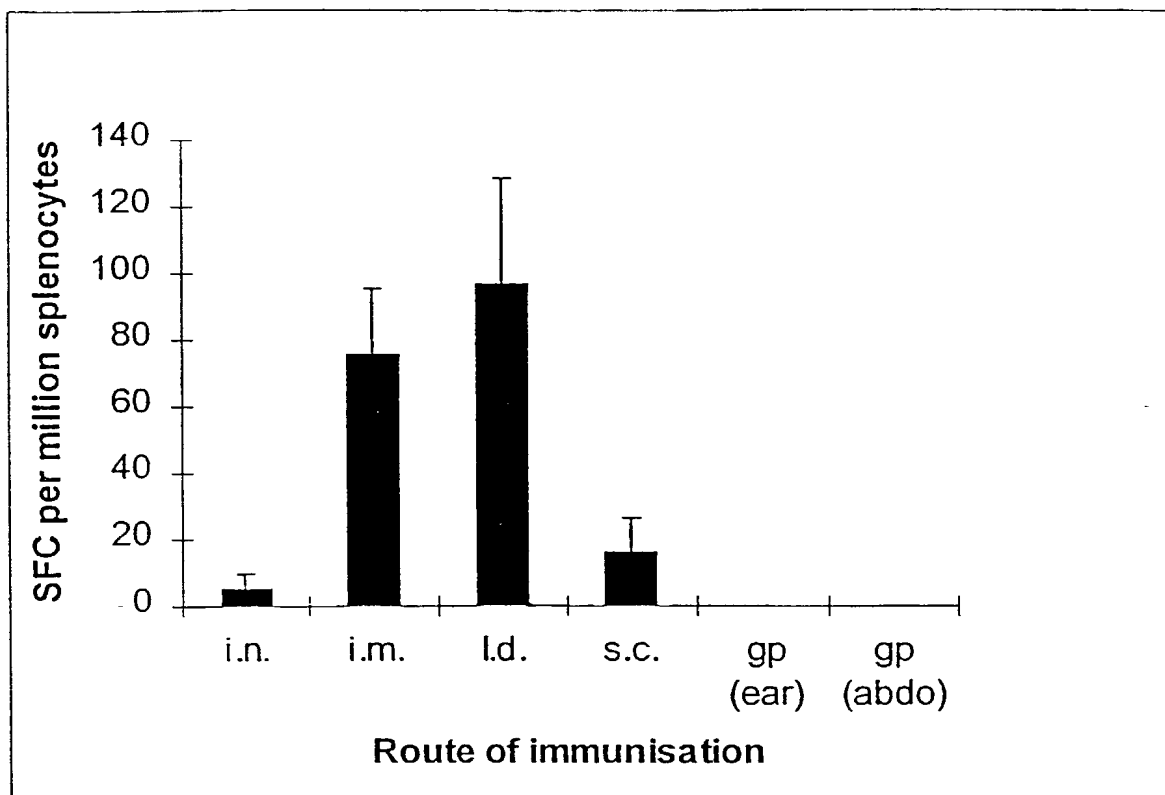


Figure 2

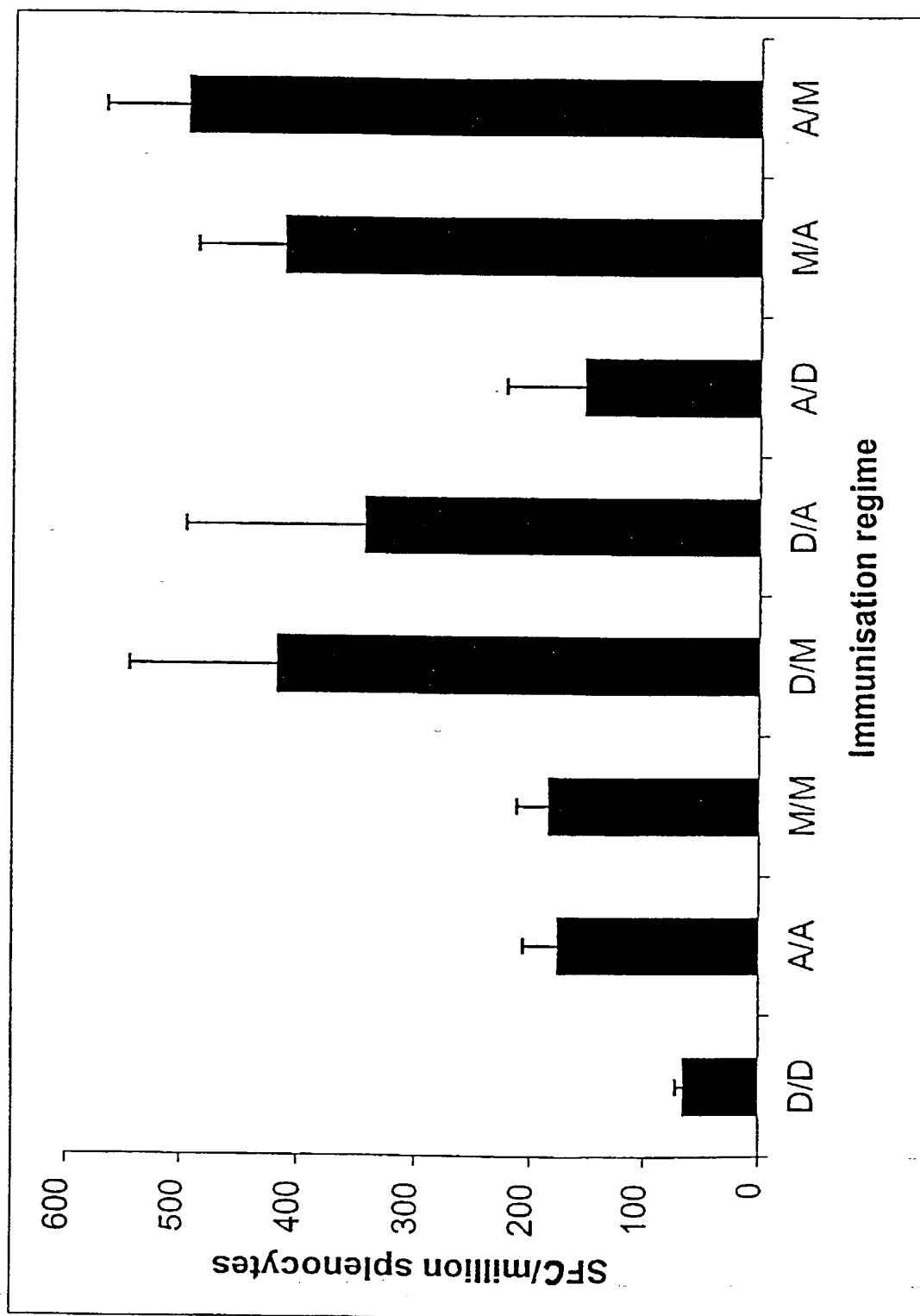
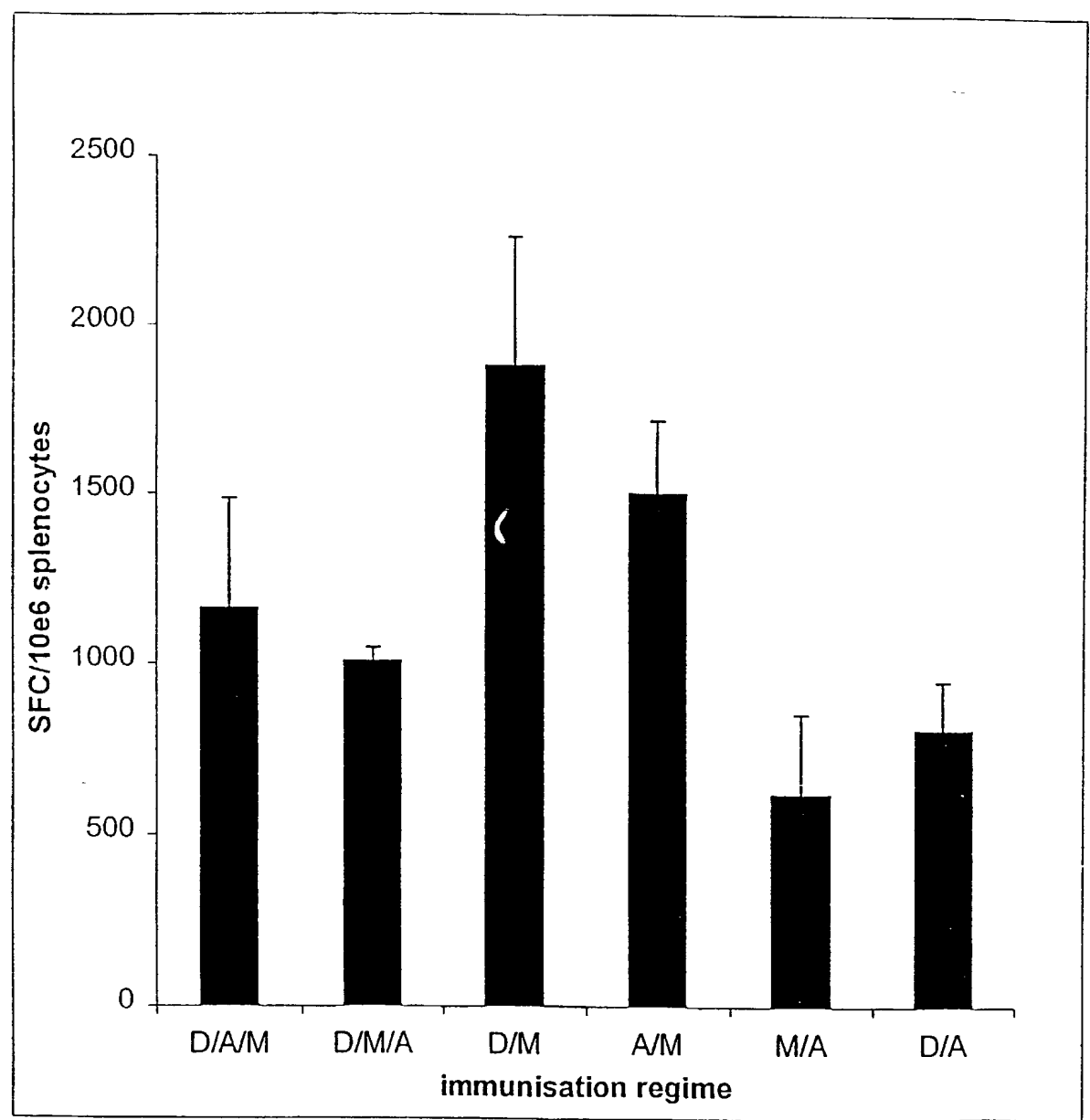


Figure 3



620-190
SMW/BP5998190Nixon & Vanderhye P.C. (10/99)
(Domestic Non-Assigned/Foreign) Page 1

RULE 63 (37 C.F.R. 1.63) ·
INVENTORS DECLARATION FOR PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

As a below named inventor, I hereby declare that my residence, mailing address and citizenship are as stated below next to my name, and I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

USE OF REPLICATION-DEFICIENT ADENOVIRAL VECTOR TO BOOST CD8+ T CELL IMMUNE RESPONSE TO ANTIGEN

the specification of which (check applicable box(es)):

☐ is attached hereto
☐ was filed on _____ as U.S. Application Serial No. _____ (Atty Dkt. No. 620-190)
☒ was filed as PCT International application No. PCT/GB00/03601 on 20 September 2000
 and (if applicable to U.S. or PCT application) was amended on 05 November 2001

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose to the Patent Office all information known to me to be material to patentability as defined in 37 C.F.R. 1.56. I hereby claim foreign priority benefits under 35 U.S.C. 119/365 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed or, if no priority is claimed, before the filing date of this application:

Priority Foreign Application(s):

Application Number
9922361.2

Country
Great Britain

Day/Month/Year Filed
21 September 1999

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below.

Application Number

Date/Month/Year Filed

I hereby claim the benefit under 35 U.S.C. 120/365 of all prior United States and PCT international applications listed above or below:

Prior U.S./PCT Application(s):
Application Serial No.

PCT/GB00/03601

Day/Month/Year Filed
20 September 2000

Status: patented
pending, abandoned

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon. And on behalf of the owner(s) hereof, I hereby appoint NIXON & VANDERHYE P.C., 1100 North Glebe Rd., 8th Floor, Arlington, VA 22201-4714, telephone number (703) 816-4000 (to whom all communications are to be directed), and the following attorneys thereof (of the same address) individually and collectively owner's/owners' attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith and with the resulting patent: Larry S. Nixon, 25640; Arthur R. Crawford, 25327; James T. Hosmer, 30184; Robert W. Faris, 31352; Richard G. Besha, 22770; Mark E. Nusbaum, 32348; Michael J. Keenan, 32106; Bryan H. Davidson, 30251; Stanley C. Spooner, 27393; Leonard C. Mitchard, 29009; Duane M. Byers, 33363; Jeffrey H. Nelson, 30481; John R. Lastova, 33149; H. Warren Burnam, Jr. 29366; Mary J. Wilson, 32955; J. Scott Davidson, 33489; Alan M. Kagen, 36178; Robert A. Molan, 29834; B. J. Sadoff, 36663; James D. Berquist, 34776; Updeep S. Gill, 37334; Michael J. Shea, 34725; Donald L. Jackson, 41090; Michelle N. Lester, 32331; Frank P. Presta, 19828; Joseph S. Presta, 35329; Joseph A. Rhoa, 37515; Raymond Y. Mah, 41426; Chris Comuntzis, 31097; Gary T. Tanigawa, 43180. I also authorize Nixon & Vanderhye to delete any attorney names/numbers no longer with the firm and to act and rely solely on instructions directly communicated from the person, assignee, attorney, firm, or other organization sending instructions to Nixon & Vanderhye on behalf of the owner(s).

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RULE 63 (37 C.F.R. 1.63)
INVENTORS DECLARATION FOR PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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the specification of which (check applicable box(es)):

☐ is attached hereto
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Priority Foreign Application(s):

Application Number
9922361.2Country
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Application Serial No.
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20 September 2000Status: patented
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